

Slime Analysis of Painted Steel Panels Immersed in Biscayne Bay, Miami Beach, Florida

G. I. LOER

Environmental Biology Branch Environmental Sciences Division

and

N. SMITH

D.W. Taylor Naval Ship Research & Development Center Annapolis, MD 21402

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O. ASSTRACT (Continue on reverse side if necessary and identify by block number. Light section microscopy was used to assess slime film formatic and dynamic exposure at a Biscayne Bay, Miami site. Both Navy Slime film thickness was measured and reported for exposures between coatings seem promising in terms of repression of slimes.	on on panels subjected to both static and commercial coatings were used. tween December 1978 and August 198

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SLIME ANALYSIS OF PAINTED STEEL PANELS IMMERSED IN BISCAYNE BAY, MIAMI BEACH, FL.

INTRODUCTION

As part of a more general study of the performance of anti-fouling coatings, a program of exposure is being carried out at the facilities of Miami Marine Research Inc., Miami Beach, Florida. The motivation for this study is to select optimum coatings for vessels with the objective of minimizing drag. In the recent past the primary criterion for successful anti-fouling coatings was effectiveness in retarding hard fouling (barnacles, tubeworms, calcareous bryozoans) and voluminous growth of macroscopic algae such as Ectocarpus and Enteromorpha. As coatings are developed which meet this goal increasingly well attention to the less conspicuous causes of hydrodynamic drag becomes important: that is, the effect of the soft fouling organisms, the microbial slime films, and the inherent roughness of the coatings themselves.

MATERIALS AND METHODS

Costings tested in this program were standard and experimental Navy materials and a selection of proprietary costings supplied by commercial manufacturers.

Navy coatings tested were F-121, F-129, and F-1020A, which are in current use, and three NSRDC-developed experimental coatings.

Proprietary information concerning the coatings is presented in Appendix C, for limited distribution.

Each coating was applied to a set of four curved sample panels which were manufactured to fit upon the circumference of a drum which was rotated at a peripheral velocity of 22 Km. The programmed exposure consisted of alternate dynamic and static periods. At the conclusion of each period, each set of quadruplicate panels was examined in a number of ways and then re-exposed. In some cases, panels were cleaned by brushing and re-examined before re-exposure. Some of the coatings failed during exposure; these were removed from the test after the next inspection. The inspection evaluated several properties. A general visual inspection by the unaided eye was made first and the results reported in terms of degree of coverage by the various classes of fouling organisms and the general apparent physical condition of the coatings. Slime film formation was more closely followed by exami-

Manuscript submitted January 8, 1981.

nation with the light section microscope; this instrument and its application to studies of microbial slime film formation are described in Appendix A of this report. After the visual initial examination, two panels from each set of coatings suitable for brushing were brushed while wet. Light section measurements of possible residual slime films were made, and the panels were then air-dried to the extent necessary to determine both coating thickness by use of a magnetic gauge and surface roughness by use of a stylus instrument. This report is principally concerned with the slime thickness measurements; the other measurements were described more fully by Acampora and Smith (1) in a report dealing with the initial observations obtained and an additional report covering the later stages of the program will be forthcoming.

As the exposure schedule and observations progressed, identification of attached organisms through use of a microscope led to the realization that such examination could yield valuable information on the physical condition of the coatings which could not be easily detected otherwise. Consequently, microscopic observations and photomicrographic records of coating condition were made as the studies progressed. These studies will be described more fully in a forthcoming joint report with Mr. Perez, of Miami Marine Research Inc.

The Slime Film Measurement

The measurement of highly hydrated films should be performed in an environment in which the activity of water is that of the medium in which the film was formed, so that the degree of swelling of the film matrix is that of the immersed film. Therefore, the filmed panel was flooded with filtered seawater, placed in a closed container together with an absorbent paper pad saturated with an excess of seawater, and the wet film thickness measured repeatedly until three successive measurements remained constant. The measurement itself, discussed in detail in Appendix A, consisted of illuminating the specimen with an image of a fine slit at an incident angle of 45° to the surface, and measuring the separation of the reflections from the top of the film and the interface of the film with the coating surface. This separation was proportional to the film thickness.

Slime Film Structure

The wide variety of microorganisms present in natural environments leads to a corresponding variety of structures when they colonize an immersed surface to form slime films. Such films vary from almost perfectly transparent to completely opaque structures even when no substances such as sand or silt are incorporated in them, due to the pigmentation of the microbial cells and the size of the refractive index inhomogeneities making up the structures. Further, some microorganisms grow in colonies which are well separated compared to their height, and so appear as clumps, while others grow in extensive coherent colonies and therefore are inherently smoother. In a natural coastal

site, such as Miami, there is also much suspended material which becomes deposited on or incorporated into the microbial colony and so affects both transparency and surface roughness of the microbial film. While in most cases the films examined for this study were sufficiently transparent for the light section method to visualize both the reflections from the top and bottom of the slime, in several cases a small region of opaque film was gently rubbed with a chisel-shaped Teflon tool and then rinsed with filtered seawater to remove opaque material and allow visualization of the bare coating surface for the slime thickness measurement. Photographs of slime films shown in Fig. la and Fig. 1b illustrate two clump forms of colonies. The reflection from the surface of the paint film is the lower, fainter line in each photo. The reflection from the upper surface of the hydrated film is the much brighter line. Colonies of slime microbes are seen in both photographs, partially obscuring the lower reflection, and spanning the thickness of the hydrated layer. These colonies are well-separated in Fig. 1a, and are more continuous in Fig. 1b.

Surface Roughness

As indicated in Appendix A, the reflections of the slit are affected by deviations from planarity. Therefore, the profiles of the surfaces in equilibrium with seawater are apparent in the observed images, and may be recorded photographically without mechanical disturbance. Although surface roughness may in principle be derived from such photographs, we have not attempted to do so quantitatively. Rather, we have presented examples of surfaces of varying roughness in Fig. 2 so that the potential of the method can be appreciated by the reader. These photographs are of samples which had been cleaned of microbial slime by brushing. Methods of visualizing slime films by incorporating fluorescent or reflective materials would allow easier analysis of the deformations and profiles of the slime films under flow situations or under mechanical loading, to relate physical properties of the slime films to operating parameters of the coated equipment. Such work may be attempted in the future.

Panel Designation

Each coating system was applied to a set of four individually numbered panels, and the groupings can be observed in the Table presented in Appendix B. The panel sets will be referred to in the remainder of this report by the lowest numbered panel in that set. Thus, panel set "1" consists of panels #1, #2, #33, and #34. These panels are described by the same numbering system in the report by Acampora and Smith (1).

The Navy coatings used in this work were F-121 (panel sets 1, 7, 97 and 101), F-129 (panel sets 3 and 9), F-1020A (panel sets 5, 11, 105 and 109), the NSRDC-developed Galcoats (panel sets 81 and 173) and other experimental formulations designated NSRDC-1114 (panel set 85), and NSRDC-OMP-A-28 (panel set 89).

The proprietary materials are defined in Appendix C, for limited circulation.

Results of the Miami Test Program to August 1980

Analysis of the slime film thickness measurements are presented graphically as Fig. 3, and in Tables I, II, and III, and in Appendix B, from which Fig. 3 is derived. The lower limit of detectability of the hydrated film upon a surface is influenced by several properties. Firstly, the color of the sample is important, because the reflection from a dark surface is weaker than that from a light one. The reflection from the upper surface is usually much more intense, and masks the reflections from the darker pigmented surfaces. It is also easier to visualize a slight separation of the reflections if the surface is very smooth. Therefore, the limits of detection of a film upon a coating surface vary from 0.3 X 10^{-3} to 0.8×10^{-3} inch (i.e., 8 to $20~\mu m$; these limits must be kept in mind when interpreting the data.

Figure 3 shows the thickness of the hydrated layer retained by the panels as the test program progressed. The data are presented as the average thickness, and standard deviation, of the hydrated layer observed upon the sets of quadruplicate panels. Numerical data from which Fig. 3 is compiled are presented in tabular form in Appendix B. Each set of four panels is referred to by the lowest panel number of the set. Panels are grouped by set in the table, and individual and set average hydrated layer film thicknesses are presented. Standard deviations within each set are also given. Where two thickness measurements are presented for a panel, the thinner refers to the hydrated layer between clumps of slime which project above the thinner general slime layer. The thicker refers to the height of a typical projecting slime clump above the opaque paint surface.

After the dynamic exposures, and after brushing, the film thicknesses were less than were found after the static portions of the exposure cycle and difficult to determine from the figure. Therefore, the following discussion and Tables I-III are presented to aid in evaluation of performance.

The panels used in this testing program were placed under test upon receipt, so no microscopic observations could be made upon their fresh surfaces. However, other panels coated with Navy F-121, F-129, and Fl020A panels were made available, and examined by light section microscopy upon immersion in distilled water and in clean artificial seawater made up from nominally inorganic constituents. These coatings were at first hydrophobic and so shed water, making measurement of hydrated film thickness futile. After soaking in artificial seawater for 24 hours, however, the coatings became wettable, and measurements of the retained film could be made. Using the criterion that three successive readings showed no decrease in film thickness, hydrated layers of up to one mil (25 µm) could be found. The signi-

ficance of such layers is unclear at present. The fresh paints bearing these films did not impart the sensation of lubricity characteristic of microbial fouling when rubbed with the fingers. As mentioned in Appendix A, these apparent hydration layers above the well-defined opaque pigmented surfaces may be highly swollen layers of paint vehicle components, or adsorbed dissolved matter, or the result of initial adherance of microbes, but no definitive statement concerning their nature is possible now. The thin hydrated layers observed on some panels after dynamic testing or after wet brushing present a similar appearance, but may not be of a similar nature. There is a need to develop methods to characterize the properties of such films to understand their hydrodynamic significance.

Surface Roughness

The observations made while measuring slime film thickness allow some qualitative estimates of relative surface roughness of the various paint films to be made. These observations, made through the retained aqueous layer on panels which were still wet, are not as precise as the stylus measurements made upon air-dried samples (1, Acampora and Smith), nor is any attempt made to assign absolute values to the measure of roughness. However, if we consider relative ranking of the coatings, on a scale of 1 (smoothest) to 6 (roughest) and admit that subjective assessments may be somewhat biased, a "rough" rank order can be presented which may be useful. Two additional features can also be observed with this technique, and are noted where appropriate. The first is checking or cracking. In several cases, patterns of cracks were evident on panels which appeared in excellent condition to the naked eye. These cracks were observed with the light section microscope, and, as it became available, with the stereo microscope. The second is what we call"scatter". This is characterized by a diffuse appearance of the band of light reflected from the paint surface, which makes the band quite broad. The significance of this broadened reflection is open to question at present. One possibility is that roughness whose lateral dimension is less than the resolution of the optics causes reflections at 900 to the incident beam to originate from a broad region of the surface. A second possibility is that the paint surface is not opaque, so that light penetrates a significant distance beneath the surface before refraction and reflection towards the observer. There may be other causes also. The observations made on brushed panels regarding relative smoothness, cracking and scatter are presented in Table I, and the relative roughness scale is illustrated in Fig. 2. The letters "C" and "S" in Table II refer to the presence of cracking and scatter respectively. The data indicate clearly that coatings 11, 109, and 105 lead to rough surfaces, while coatings 81 and 173, and perhaps 89, lead to quite smooth surfaces, and coatings 25, 153, and perhaps 137, seem susceptable to crack formation on the basis of the data currently on hand.

Self-polishing coatings hydrolyze on immersion, so as to prevent gross roughening while maintaining a good toxin leach rate. Although

these costings were not brushed, the degrees of smoothness could be estimated and are at 2-3 on the scale of 1-6.

Experience has shown that very smooth coatings do not drain as rapidly as rough ones, presumably because the channels in a rough coating enhance flow of liquid from higher regions. The time available for inspection of the large number of panels involved in this study precluded very long waits for equilibrium, so that determination of the final thickness of the hydrated layer on the panel sets 29, 81, 89, and 173 was more subjective than on the rougher coatings, and may be biased toward higher values.

Observations on Panel Sliming

Figure 3 is a visualization of the data obtained at the Miami Beach test site from Dec. 1978 until July 1980. Several observations may be made concerning the data.

The first set of observations was made at the conclusion of a dynamic cycle in December of 1978. All the observations taken at that time indicated a significant hydrated layer, of the order of one to two mils thickness. As it had been found that new paints showed that effect after static exposure in the laboratory, it was not entirely unexpected at the time. However, when sets 85, 89, 105, 109, 121, and 125 were immersed for their first dynamic cycles, the film thickness measured was less, although #81 (a very smooth coating) showed a significant film thickness after first dynamic exposure. These observations may reduce the significance of the measurements of Dec. 1978; perhaps drainage had not been complete. Note also that there seems to be a seasonal maximum during the May-August 1979 period. For convenience the hydrated layer thickness after the first dynamic cycle, and the thickness after the dynamic cycle yielding the greatest thickness for the particular panel set, are shown in Table II.

NAVY COATINGS

The Navy coatings used were of 6 types: F-121, F-129, and F-1020A, which are in current use and were used with vinyl or epoxy undercoats, and Gelcoat OMP-A28 and NSRDC 1114, which are NSRDC-developed experimental coatings. There are clear differences between these. The F-121 and F-129 are copper antifoulants, and show better control of the sliming which forms under static conditions than the organotin F-1020A. The F-129 and F-1020A show smaller films after dynamic cycling than F-121. Thus, the F-129 coating seems promising in both respects. This preference is reinforced by the great wear rate observed with F-121. If only dynamic performance is at issue, F-1020A is also a good performer. The Gelcoats are very smooth coatings, and so the steady value of very low film thickness is somewhat subjective as previously mentioned. OMP-A28 was not as smooth intially, but became quite smooth as the program progressed (Table III). NSRDC-1114 failed

early and was discontinued. These organotin coatings were heavily colonized, as were the F-1020A panels, during static cycles. During the last dynamic cycle observed, (June 1980) OMP-A28 (set 89) and the older Gelcoat (set 81) appeared to be maintaining a thicker film than during the previous ones. It is not yet clear whether this signifies the onset of deterioration.

Proprietary and Commercial Coatings

A variety of coatings from commercial sources were also subjected to the test program. Some comments concerning these follow:

Some coatings developed a pattern of small cracks, which could be seen easily under microscopic observation. These were 25, 137, and 153. The significance of the cracks for performance of the coatings is as yet unclear. Roughness estimates are not made at cracks, but rather refer to the regions between them. Several coatings maintained quite thin films on dynamic exposure over several cycles: sets 19, 133, 137 and 145. Sets 129, 141, 149, & 157 maintained thicker films on the last dynamic cycle. Sets 17, 19 and 25 were on test for a long time (6 cycles), which in itself is a recommendation because badly fouled or otherwise damaged panels were removed from the test when noted at the scheduled inspection. Slime film thickness of set 19 appeared to be maintaining performance except for a slight increase of film thickness during the last dynamic cycle (June 1980). Set 25 was beginning to show cracking. Sets 153, 161, & 165, showed very low dynamic film thicknesses, but like sets 149 and 159, were only immersed for two cycles. The slime formed on sets 141, 145, 153, 165 and 169 deserve special mention. These formed heavy cohesive films in the later static cycles. The films showed a 3-layered structure and the top layers could be pulled off in small sheets. The hydrodynamic significance of this type of film is as yet unclear.

Effects of Brushing

Conventional antifouling paints become inactive because toxin near the seawater-paint boundary progressively leaches from the seawater, leaving a depleted region. Alternatively, deposits may block leaching of toxin. In either case, brushing of the paint surface may serve to rejuvenate the coating by removing the inactive barrier to further toxin release. If toxin release is indeed enhanced in this way, it might be expected that subsequent fouling would be retarded on brushed samples in comparison with unbrushed samples. On looking through Appendix B, it may be seen that several sets of panels on occasion indicated such a difference, eg: #5 (May 1979) #9 (Dec. 1979) #89 (Aug. 1979) #105 (Dec. 1979) #105 (March 1979) #137 (March 1980) but the author does not consider the evidence strong enough to warrant the conclusion that brushing inhibits future sliming, especially on noting #17 (May 1979) and #129 (Dec. 1979) which were not brushed show similar differences in slime thickness.

CONCLUSIONS

- 1. A general summary of sliming performance is given in Fig. 3.
- 2. The Navy copper-containing coatings show significantly less sliming under static conditions than organotin coatings.
- 3. The self-polishing coatings yield wet roughness indices of 2 to 3, whereas other coatings range from 1 (smoothest) to 6 (roughest).
- 4. Differences in wet roughness as well as sliming should be taken into account in assessing paint performance.
- 5. Optimization of coating choice requires better understanding of the relative contributions to drag of roughness, slime thickness, and the viscoelastic properties of the microbial slime films and the coatings themselves.

ACKNOWLEDGMENT

Many of the measurements were made by Mr. Brent Wright of Miami Research Inc.

REFERENCE

 Acampora, M. and N. Smith. Screening Test for Selection of Smooth Hull Coatings Through Alternate Dynamic and Static Exposure at a Natural Fouling Site in Biscayne Bay (Miami Beach, FL). Report #SME-80-14, David W. Taylor Naval Ship R&D Center, Annapolis, MD.

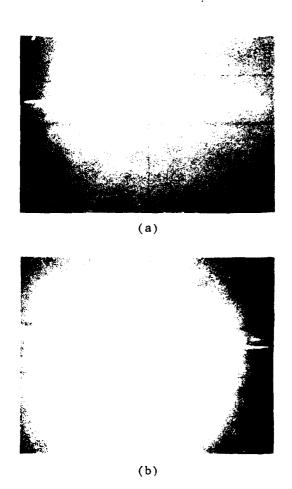


Fig. 1. Slime Film Colonies Visualized via Light Section Microscope.

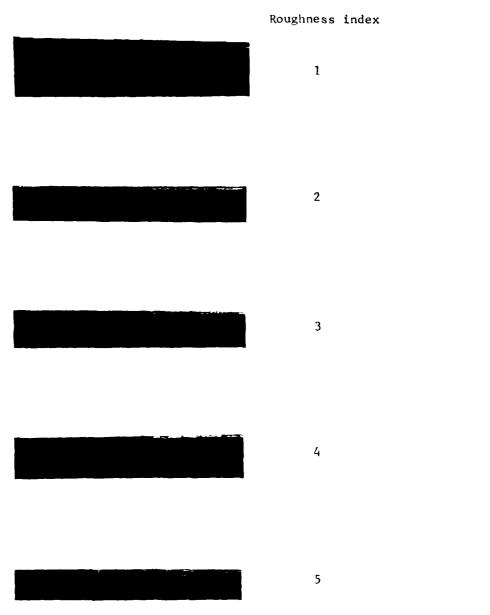
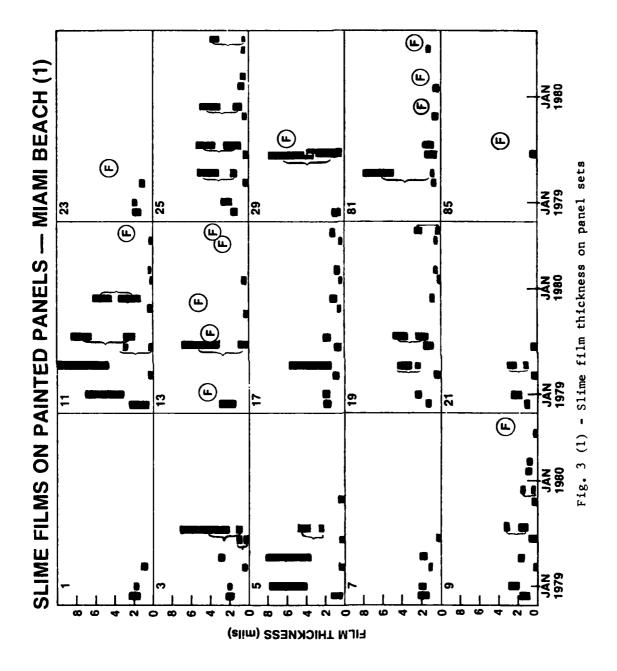
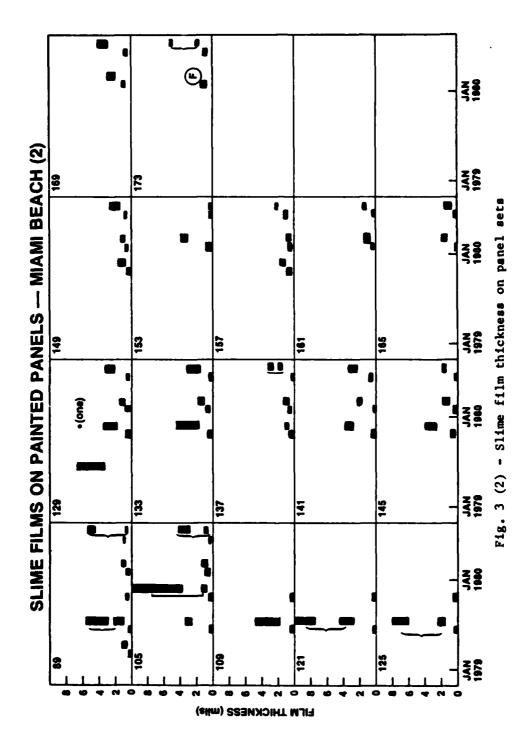


Fig. 2. Brushed Paints Visualized via Light Section Microscopy. (Numbers indicate surface roughness index)





Surface roughness of brushed panels. Roughness ratings: I (smooth) to 6 (roughest) {
Surface cracks denoted by C }
{
Diffuse scatter denoted by S } TABLE I.

Pane 1	Set i	Panel Set # 3 5	2	6	111	13	25	29	81	88	105	109	137	153	173
Date:															
Aug. 79	62	6	m	s 3	2	6	8 8	6		m	e	•			
Nov. 79	19			4	\$	6	0		7	7	y S	S	7 S		
Feb. 80	26			6	9	6	4	6	,	6	9		6	c 3	-
Mar. 80	980			٣	9		7 0	6	7	-	4		~		
June 80	2			80	9	7 S	9 0	-	-	-	S S		7		-
July 80	2						c 3				8 3		C 2		

TABLE II. Average thickness for set of 4 panels after dynamic exposure for (a) first dynamic exposure and (b) for the exposure other than the first yielding the maximum average thickness.

	Thickne	ess after
Panel Set	First Dynamic	Max. Dynamic
1	2	1
3	2	1
5	1	DL
7	2	1
9	1½	ļ.
11	11/2	3 1 1
17	2	1
19	14	1
21	1	DL
23	2	1
25	1½	1/2
29	1/3	Ţ.
81	3/4	1
85	DL	F
89	DL	1
105	DL	1/2
109	DL	DL
121	DL	DL
125	DL	DL
129	5	<u>1</u>
133	DL	DL
137	DL	Ł
141	DL	1/2
145	1/2	DL
149	DL	0.7
153	4	DL
157		1
161	1/3	DL
165	DL	DL
169	1	2/3
173	i,	3/4
- · ·	•	

Here the entry "F" indicates failure of the coating system. the entry "DL" indicates no film thicker than detection limit.

TABLE III. Film thicknesses greater than detection limit where found after brushing.

Panel Set	5	9	13	25	29	81	89	105	109	137	153	173
Date												
Jan.	-	-	-	-	•	-	•	-	•	•	-	-
July 🕴	.5		•4									
Aug. 5			.4	.4	.3	.4*	•4					
Nov.	}				.3	•5	.5	•5	•5			
Dec.		.5	.3	.4	.6	.8*	.6	.4				
Feb.		.8	.4	.8	.3	.4	.4	.4		.4	.4	.8*
Mar.0	1	.4	.4		.4	.5*	.6*			.4		.5*
June		.3		.6		.8*	.5*			.4		.9*
July	ł							.5		.4		.4*

^{*}Indicates smoothest surfaces (See Table II).

APPENDIX A

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Measurement of Microbial Marine Fouling Films by Light Section Microscopy

George I. Loeb Nevel Research Leboratory Washington, DC

A non-destructive optical method of surface inspection has been applied to measurements of the thickness of marine microbial slime fouling films. Application is described to fouling of metal heat exchanger pipe in the Ocean Thermal Energy Conversion program, and to slime formation on antifouling paint samples. Films greater than $8\,\mu m$ in thickness may be detected with precision of $3\,\mu m$ in feverable cases using our instrument; other instruments are also available. An important feature of the method is its possible application in measurement of roughness of surfaces not amenable to stylus instruments, such as soft materials and immersed surfaces.

INTRODUCTION

The efficiency of equipment operated in aqueous environments is often affected by formation of microbial fouling, or slime, films on their surfaces. Heat flux across heat exchanger surfaces,' the operation of trickling filters,3 and resistance to hydrodynamic flows are examples of processes in which microbial colonization of Immersed surfaces are thought to be important. Further, it has been suggested that microbial films influence colonization and development of the larger members of the fouling community," such as bernecies, bryozoa, and tubeworms, which are generally assumed to degrade the operation of many kinds of equipment. The ability to measure silms film layers nondestructively, then, is of value in studies of the function of marine equipment. This communication describes the application of one such technique: light section

Before the light section instrument became commercially svaliable other methods for determination of alime layer thickness were developed. Kenis * et al, Hoshn & Ray,* and Charactilis * have described the use

of an electrical method in which a micrometer-driven probe is made to approach the surface, and contact is signalled by a decrease of electrical resistance. This method requires a conductive contact to the slime to complete the circuit, and mechanical access to the silmed surface. Characklis ¹ also describes volumetric measurements after a period of drainage of samples of known surface area to determine slime thickness; this requires a sample which can be immersed in volumetric vessels. Measurements of the distance of focus levels of the uppermost portions of the slime from the substratum using a microscope have also been made.5, 5 This method does not require mechanical contact, but requires that many points be measured individually in order to visualize the nature of a reasonable area of film, and does not present an easily interpretable indication of surface contour. The light section technique combines the advantages of a non-destructive contact-less thickness measurement, with applicability to a wide variety of substratum materials and an easily interpretable depiction of surface profile.

The principal of the light section technique has been presented by Schmeltz * and developed by Tolensky, * who has extended its applicability to the dimensions of single cells. Here, however, applications making use of commercially available equipment only are described.

Note: Mention of specific products or manufacturers dose not constitute recommendation by the Dept. of Defence.

CORRECTIONS

Eq. 3
$$d_{\phi} = 2 g_{\phi} \cos \theta = 2h \tan \theta \cos \theta$$

Eq. 4
$$\frac{d_{\phi}}{d_{d}} = \cot \phi$$

Eq. 4
$$\frac{d_{\phi}}{d_{\phi}} = \cot \phi$$
Eq. 5 $\sin \phi = \frac{n_{\phi} \sin \phi}{n}$

Eq. 6
$$\frac{d_{\phi}}{d_{\phi}}$$
 = cot $\left[\operatorname{arc sin} \left(\frac{0.707}{n} \right) \right]$

CAPTION ERRORS

Fig. 2. should read "at step"

Fig. 6. Should read: Cover slip thickness is (B-A)-(C-D)

Refractive Index may be obtained from the ratio

$$\left\{ \frac{(B-A)-(C-D)}{(B-C)} \right\}$$

Fig. 7. should read "covered Sample Container".

I am sorry that the journal's editorial policy did not allow proofreading by author. Better photographs may be requested if desired.

The Light Section Technique

The original form of the instrument consists of two metched compound microecopes, oriented at right engles to each other (Figure 1). An illuminated slit is placed at what would be the normal eye position of one microscope, and a reduced image of the slit is focused via its objective onto the specimen. The specimen is positioned with its normal bisecting the angle formed by the optic axes of the two microscopes to capture maximum reflected light, and with the slit image on the specimen surface in sharp focus via the second micro-**20008**.

If the specimen surface is flat, then the observer sees a straight line slit image. Any deviation of the specimen surface from planar, however, causes a corresponding deviation in the slit image. The magnitude of the observed deviations may be measured; for example, by a filar micrometer or on a photographic image.

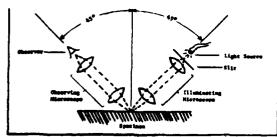


Figure 1. Schematic Diagram of Light Section Microecope.

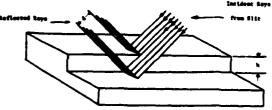


Figure 2. Path of Rays Parallel to Optic Axes as Step in Specimen, d is observed deviation due to step of height

The nature of the deviation caused by a step on the surface is shown in Figure 2. The intersection of the slit image with the bottom level is further from the light source than that with the top level, so the portions of the slit image at the two levels appear to the observer as line segments displaced from each other by the distance "d" as also shown in Figure 3-1. The case of a nonopaque layer upon a second material is similar. In this case, as shown in Figure 3-II, two lines are observed: one from the upper and one from the lower interface, but the observed displacement depends upon the refractive index of the layer.

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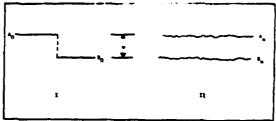


Figure 3. Diagrams of Light Section Microscope Visual Fields from (I) the step of Fig. 2 and (II) a microbial film on a painted surface.

The sources of the reflections represented are:

Ac: upper step surface

Bo: lower step surface

An: upper film surface

Bn: film-paint interface

The relations between the step height or layer height and the observed displacement may be shown with the aid of Figure 4 and a treatment similar to that of Mansour. A light beam from the slit S is partially reflected from the upper level at A, and partially from the lower level at B. if the specimen consists of a step surface. then the ray path shown by the dashed line represents the lower surface reflection. (These upper and lower reflections may be related to the ray indicated by the double arrow in Figure 2.) The observed displacement is in this case $d_{\xi} = 2 Z_{\theta} \cos \theta$, where $Z_{\theta} = h \tan \theta$. (1)

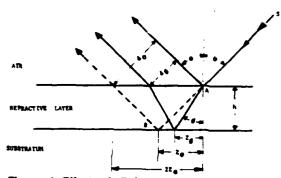


Figure 4. Effect of Refraction on Apparent Film Thickness.

Therefore,

 $d_{\theta} = 2 h \tan \theta \cos \theta = 2 h (0.707) \text{ for } \theta = 45^{\circ}.$ (2) If h is the height of a non-opaque layer of refractive index n, then a ray impinging at A will be partially reflected at the upper level, and partially reflected at the lower level, with refraction at the upper level for both passages through the interface. The displacement observed between the upper and lower reflections will

$$d_{\theta} = 2 Z_{\theta} \cos \theta = 2 h \tan \theta \cos \theta \qquad (3)$$

and if $\theta = 45^{\circ} =$

$$\frac{d_{\theta}}{d_{\theta}} = \cot \theta \tag{4}$$

The law of refraction yields:
$$\sin \theta = \frac{\log \sin \theta}{\Omega}$$
 (5)

Where n_0 is the refractive index above the non-opaque layer. For air at $\theta=45^\circ$, $n_0=1$ and ain $\theta=0.707$ so that

$$\frac{d_{\theta}}{d_{\theta}} = \cot \left[\arctan \left(\frac{0.707}{n} \right) \right]$$
 (6)

Thus, the displacement caused by a thin refractible layer upon a surface in air must be multiplied by this quantity to yield the displacement caused by a step of equal height. This quantity is plotted as a function of n in Figure 5 and is 1.61 for a layer of seawater, in agreement with the calculation and calibration by Mansour.

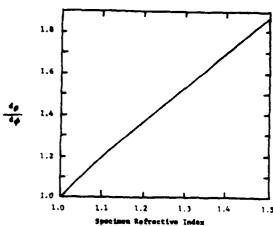


Figure 5. Correction Factor for Refraction of Non-Opaque Layer.

Other methods of achieving the light section effect have been developed using specialized optical components, 7. * but the work described here was performed with a Gaertner Model M-308 instrument,18 which is one of the type shown in Figure 1. The objectives are 3.5X, and a filer micrometer-10X eyepiece is supplied. The instrument is direct reading in that the filer micrometer measurement of de in air is equal to the step height of step specimens measured with a machinist's micrometer. (A microscope slide cover slip is a good test object, but if an air space is noticed under it during the light section measurement, the thickness of the air gap must be subtracted from the total step height: Figure 6. Other manufacturers 11, 12 also supply light section instruments of this general type with greater magnification but with less working distance than the Gaertner instruments.

Application to Marine Microbial Silmes

When a solid which had been immersed in a natural water system is recoverd, a highly hydrated microbial slime film may be found adhering to its surface, if, dur-

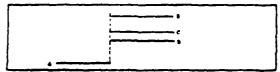


Figure 6. Diagram of Light Section Visual Field Resulting from Edge of Cover Ship on a Flat Surface. The sources of the reflections represented are:

- A) The bare flat surface B) The top of the cover slip
- C) The bottom of the coverD) The flat surface under slip the cover slip

The cover slip thickness is (B-A)-C-D). The refractive index may be obtained from eq. (6) and the ratio

$$\begin{cases} (B-A) - (C-Df) \\ (B-C) \end{cases}$$

ing drainage of excess liquid, the solid is kept in an atmosphere which is in equilibrium with the natural water, then the activities of the solvent components in the film are the same as in the natural water, and the slime film remains hydrated. We take the thickness of the hydrated layer to be the slime film thickness, although the surface tension at the air-microbial film interface may result in a somewhat smoother slime film boundary than is present during immersion. (This assumption has also been made by workers using the electrical probe and volumetric methods 2. 3. 4). Since microbial slimes are usually more than 90 percent hydrated * the refractive index of seawater may be used to determine thickness. Films of composition appreciably different from seawater, such as hydrated corrosion product films, require an estimate of refractive index before thickness can be determined. It may sometimes be possible to circumvent this requirement by a light-section measurement at the edge of a cleaned region on the surface where the refractile film may be treated as a step, or by measurement of a sample of known film thickness. Refractive index may in some cases be inferred if the sample composition is known.

The activity of the medium at the sample surface may be conveniently controlled by allowing the sample to equilibrate with a reservoir of the medium in which it was grown inside a closed container. A long working distance instrument is very convenient in this type of application.

Accuracy, Precision, and Image Quality

The accuracy of measurement may be checked by determinations made by other means. Mansour has measured the thickness of coatings and thin plastic films with a Zeiss light section instrument, comparing the results with oblique section and micrometer measurements. Our instrument has been calibrated by observing the thickness of cover slips for microscope slides also measured with a machinist's micrometer. This calibration must be performed when any change in

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the effective tube length between the filar micrometer at the eyepiece and the objective is made. While such changes need not be made if only direct viewing of dry samples is done, observation of wet films in humid environments requires observation through transparent windows. Clear viewing through windows may require slight modifications of the eyepiece and slit to objective distances, so that recalibration of the filar micrometer is necessary when the window is changed.

The precision of a film thickness measurement depends upon the ability to superpose the filar micrometer hairline upon the stit image. This, in turn, depends upon the thickness and definition of the slit image as observed upon the specimen surface. It is observed that bright metallic surfaces, or reflective surfaces such as glass, may be discerned at quite low sirt widths and light intensities, and therefore located with good precision even when close to the highly reflective upper surface of a hydrated layer. The lower limit of measurement for the hydrated layer thickness is then~8µm and films may be measured with a precision of ~3µm. For rougher surfaces of lower reflectivity, and darker colors, the presence of the bright reflection at the upper surface of the hydrated layer makes the lower limit somewhat larger (~12-15µm), but as the film thickness increases, the precision of the measurement is also approximately ~8-4µm. In order to optimize the visibility of the filar micrometer a small variable intensity lamp is very helpful for faint illumination of dark samples. A polarizer at the eyepiece is often useful to preferentially attenuate the bright upper reflection. Photography through the eyepiece has been found feasible with a single-lens reflex 35 mm. camera fitted with a 50 mm. standard lens-close-up lens combination, as was suggested by the manufacturer.

As the thickness increases beyond $500\mu m$ (20 mils) the precision of measurement decreases, since both surfaces cannot be in focus at the same time; however films of~40 mils thickness can be measured to \pm 1.5 mil (~87 μ m) in favorable cases.

APPLICATIONS

The light section technique has been used in two recent marine microfouling studies, on samples of quite different character.

The first group of samples was studied as part of the Ocean Thermal Energy Conversion (OTEC) program. Fouling of heat exchangers is of vital importance to this program, since the temperature differences driving the process of power generation are so small that small decrements in efficiency are serious! A series of exploratory tests on materials under consideration for heat exchangers was undertaken to assess the various design options. Samples of tubing which had been exposed to flowing seawater in the Gulf of Mexico for various times were retrieved and subjected to a battery of physical, chemical, and biological tests. As part of the test program wet film this ress was measured.

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Details of the preparation of these samples have been presented elsewhere.13 Briefly, they are approximately V2-inch coupons cut from the 1-inch metal pipes and preserved in glutaraidehyde (4%)-seawater medium. To achieve equilibrium hydration of the fouling film, the samples were placed on discs of filter paper saturated with a slight excess of the medium in Petri dishes with tight friction-fit covers (, con plastics #1006). A capillary path for liquid between the sample and the reservoir of medium in the dish was provided by a length of well-washed white cotton thread laid across one edge of the sample and in contact with the paper. Any debris on the sample surface was removed by gentle rinsing with medium ciarified by sedimentation. Wet film thickness was determined after equilibrating overnight at 4°C, then for two hours at ~25°C, after which no changes were observed for several hours.

The measurements made on OTEC samples, and described previously,12.14 showed increasing values of film thickness with time, and different materials showed different fouling behavior. Aluminum rapidly developed a rather thick film which increased to about 20µm. Independent data from chemical analyses of replicate samples 13 showed that a corrosion film formed rapidly, and that when little biofouring was found by scanning electron microscopic examination, the wet film thickness from light section microscopy could be accounted for by the corrosion product present. A thicker wet film was found when biological material was present, and the increase in thickness correlated well with the thickness of the biological material found by S.E.M.—i.e., 10µm. Titanium and AL6X stainless steel tubes developed hydrated layers more slowly which were closely correlated to microbiological growth observed with the S.E.M. rather than to corrosion films. The fourth material tested was a cupro-nickel alloy. Corrosion of this material resulted in a dark granular product, whose outer layers were easily dislodged by even very gentle agitation during transport. It was therefore not surprising that a stable microbiological hydrated film was not found at the surface of the residual oxide by either light section or S.E.M. examination.

The second study involving this technique, which is still in progress, deals with a series of antifouling paint systems which are immersed at a test site in Biscayne Bay,16 for alternate periods of stationary and dynamic exposure. In the dynamic mode, the panels are attached to the periphery of a drum rotating with a peripheral speed of 15.5 knots. Hydrated layer measurements are made with the samples in covered trays equipped with windows at right angles to the incident and emerging light beams, as shown in Figure 7. Loose debris is rinsed from the observation area with filtered test site water. Since the sample panels are curved to fit the drum periphery, excess liquid drains from the convex surface of the panel to the adsorbent paper on the tray bottom, and measurements are made when a steady value of hydrated layer thickness is obtained (usually within 15 minutes). Although these painted surfaces are duil in finish rather than specular, and vary in color, even

black paints may be observed if the ambient light intensity can be controlled. A darkened room and a small shielded lamp whose intensity may be continuously varied to allow the proper background illumination for the filer micrometer, and a polarizer at the eyeplece to attenuate the reflection from the film-air interface are all useful.

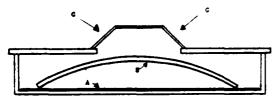


Figure 7. Cover Sample Container for Large Curved Test Panels.

S—Sample Panel A—Adsorbent Pad on Tray Floor G—Glass windows perpendicular to light beams

Microbial films are not usually opaque, and the substratum surface may be seen through them. If a considerable amount of silt is present, however, the lower reflection may not be visible. In this case, a small area of the film may have to be removed to allow visualization of the substratum and comparison with the adjacent slimed area: this occurred with several of these panels. The results of this study will be published in detail elsewhere, but we may point out here that for weakly effecting surfaces, a compromise must be struck between high light intensity and slit width to make the paint surface reflection visible, and resolution of the upper and lower reflections. For black paints, therefore, the detection limit for hydrated layers was 12-14μm (0.5 mil) with this equipment.

Results obtained with the anti-fouling paint samples indicated that, in general, static immersion resulted in considerable slime formation: films of thickness up to 250µm (10 mils) have been observed on anti-fouling panels after 30 days of static testing. After the dynamic periods, however, slimes were not evident on some panels, and only thin films on the others.

The significance of very thin films (25µm or less) is unclear at present. Some fresh marine paints show stable transparent layers of this thickness or less at high humidity after only very short periods of immersion, but do not impart the feeling of lubricity characteristic of microbial fouling. This apparent hydration layer above the well-defined opaque pigmented surface may be a highly swollen layer of a paint vehicle component, but no definitive statement as to its nature or origin may be made at this time. Thin hydrated layers of similar appearance and lacking a slimy feeling are also observed on some of the painted panels after dynamic testing. When layers of greater than 25µm are found, however, slime films are evident and accompanied by the appropriate slimy tactile sensation.

Surface Roughness

If the surface examined is not planar, the observed slit image is not linear. Thus, the image is similar to a profile of the surface. Present commonly used automated techniques for roughness measurement require that a stylus be passed along the surface, and its mechanical motions translated into electrical signals, which are processed to yield roughness parameters. Stylus techniques are not now used on immersed surfaces, however, and the distortion of soft, deformable material by the stylus raises questions in the interpretation of data. A further problem is the damage caused to some surfaces by the stylus. The light section approach yields a surface profile without mechanical contact of immersed or easily damaged surfaces. Photographic records of the light section profile may be obtained, but, at present, no commercial automatic equipment is available to analyze such records in terms of the commonly used roughness parameters. It is easily recognized, however, that some paint surfaces are rapidly roughened, as shown in Fig. 8a while others remain quite smooth (Fig. 8b).



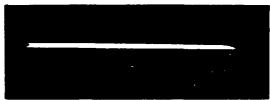


Figure 8. Photograph Through Light-section Microscope of Painted Test Panels Showing a) Cavities after Dynamic Exposure Cycle, and b) Smooth surface of different formulation subjected to the same cycle.

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George J. Loeb Is a research scientist Interested in biological attachment and surface modification. He is currently with the Naval Research Laboratory in their Environmental Blology Branch of the Environmental Sciences Division. He received his Ph.D. from Cornell University.



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Appendix B

Measurements of Slime on Panels

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Appendix B Measurements of Slime on Panels

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Appendix 8

Measurements of Slime on Panels

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**												

**Clump to 18

B-3

Appendix 8

Measurements of Slime on Panels

yluc	:	:	:			•		.5-5.0	0.5-5.0	. 5-4.9 3) (0.2)	.7-2.4	0.4-6	.9-3.7	.1-4.0	0.9-3.5 0.2) (0.8)	:	:	:			!	:	:		
June	:	:	:							0.8 (0.03) (0.03)	4. 9	4.	DL 6 .4 0	6 .3	0.4 0 (0.03) (0.		:	:			:	:	:	:	
March	÷	:	:			1.03	1.08	1.05	.60	1.02 (0.03)					0.98 (0.08)	:	:	:			į	:	:	:	
Feb	;	;	:			0.40	0.40	0.39	0.48	0.42	0.51	0.40	0.39	0.42	0.43		:	;			i	:	:		
Dec	:	:	:			•	۰.	u.	.	<u> </u>					1.0-7.4				:		;	:	:	:	
Nov	;	;	;	***		ಕ	pr3	ದ	. 38	0.34	l						ದ		ъ		ಕ	Ŀ	.45	LA.	0,4-F
Aug	;	:	:	•		1-4	0.6-2.6	2.5-3.3	1.9-7	1.5-4.2	3.6	2.5	3.1	3.0	3.0 (0.3)	4	2.2	5.4	2.0	3.3 (1.5)	4.8-7.0	2.7	4.2-12	2.7-8.8	3.6-9.2 (0.9) (1.8)
yluç	;	;	;	:		0.4	60.3	0.4	<0.3	0.3 1.3	<0.5	6.5	60.5	60.5	<0.5	9	ਵ੍ਹ	9	< 0L	10 >	0.5	9.0	4.0>	0.5	0.5 (0.06)
May	i	:	:	;		0.85	0.70	1.73	0.34	0.0		i	;	:			;	;			:	;	:	:	
March		o rough	0.5	9.0	0.5-F	0.3	0.3	0.3	0.5	<0.4 (0.1)		:	:	:			:	:	:		:	:	:	:	
1979 Jan	;		:				:						•				;	:	- 1		:	:			
1978 Dec	•	:	;	;			;	:	:			•	: :	;			:	;	i		 	:	;	;	
Pane 1	85	3 85	2	3		89	8	? ≈	26		TOR	2	32	200		100	110	111	112		121	122	123	124	

B-4

Appendix B

Measurements of Slime on Panels

yint	:	:	:	:		:	:	;	:		1	2.5	0.1	3.1	1.7	(0.8)	T.6-3.7	1.6-3.0	1.9-3.2	1.8-2.3	1.8-2.9	7.2) (0.4)	3.1-3.2	2.0-2.8	2.8-3.0	2.8-3.2	2.6.3.0	(0.5) (0.5)
June	:	:	:	;		0.42	0.47	0.47	0.4	0.45	(0.02)	.	\$	\$	Ð		E E	ş	Ş	Š	9		ŀ	S .0	0.53			
March	:	;	:	:		51:1	1.13	1.15	1.21	1.15	(0.03)	1.89	1.60	1.63	9:1	(0.3)	89.0	9.0	0.76	0.76	0.70	(0.05)	66.0	.9	0.70	1.23	1.0	(0.5)
Feb	i	:	i	;		5	ಕ	ಕ	ಕ	ಶ	45.0	<0.37	0.39	9.38 8.0	0.38	(0.1)	0.39	0. 1 0	0.40	0.39	0,40	(0.01)	N N	ದ	ಕ	ಕ	8	
) Dec	į	:	į	;	ř	\ :-	2.4-6	8.2	3.5	2.6-6.4	(0.8)	2.8	2.9	3.0	2.85	(1.3)	1.0-13.0	6.0	0.5	8.0	0.8-13	(0.2)	3.4	3.8	8.2	5.9	3.2	(0.5)
Nov	0.51	;	0.54	:	0.53 (0.01)	5	ద	ಕ	ದ	8		ಕ	ದ	ದ	8		7	ದ	ಕ	0.62	DL-0.6		ಕ	ದ	ದ	ದ	5	
Aug	•	2.5-8	1.6-6	:	2.0-7	:	:	;	• • • •			:	:	:			:	:	:	:			•	:	:	•••		
ylv	0.39	0.45	0.33	6 0.3	0.7 (0.1)	:	:	:				:	:	•			:	:	:	:			:	:	:	•		
May	:	:	:	:		:	:	:	•			:	:					:	:	:			:	:	:			
March	÷	:	;	•••		:	:	:	:			:	:				:	:	:				:	;	:	•••		
1979 Jan	:	:	:				:	:			:	;	:				:	:	;				:	:	:	:		
1978 Dec	:	;	;	***		:	;	:	:			:	:				:	:	:				:	:	:		,	
Pane 1	125	126	127	128		129	8	131	132		133	134	135	136			137	82	139	140				142	1	74		

Appendix B Measurements of Slime on Panels

	711	4	1.2	1.7	1.7	1.6	(0:5)	2.9	1.8-2.0	٠ <u>.</u>	1.5-1.6	0.5-6.1	(0.7) (0.6)	4	z	. z	i z	\ \$	₹		2.2	2.3	2.1	2.2		2.2	(0.1)	7:7	7.	₹:	1.6	-	(0.2)		
	June		ಕಕ				- 1					١		١				١		(0.3)	1.11	2	2	:	***	0.1	3	-	đ	đ	ła	ś	Ś		
	March		38	32			(0.5)	86.0	1.05	02.0			96		P .	7	~	3.7	7.5	(0.3)	99 0	9	8.5	3,	0.0	77 0	200	100:00	76.1	9:-	97.		9. i	161.01	
	1980 Feb		ದಃ	≓ ∂	s a	¥ 2	±	13 0		6.0	8 9	200	5 . 6	6.6		0.51	9.20	0.53	22 0	(0.05)	1					1		- {					0.43	(0.05)	
	٤	ś	3.6	3.7	9.6	2:3	m ć	7.3)-o.o.	c-0-1	1.6-8	1:1	1.1-5	(0.4)	:	;	;					7.5	0.9-1.3	1.3-1.9				(0.4)	:	:	;	:			
	1	AOM	Dt. (.51)	ಕ	;	0.69	0.0-0.6		.67	8.	Dr38	و د . و	0-0.5	(0.1)	<u> </u>	;		;				2.	42	4	44	5	9.0	(0.1)	:	;	;	;			
		YMY.	:	:	;	;				;	;	;				•	•	;	:			:			:	:				; ;	1	:	:}		
E Surements of		July		;	;	:				} ;	:	:				:	:	:	:				;	:	;	:				:	:	:			
ž		Max		:	:	;				:	;	:	:				;		1				:	;	;	;				:	:	:			
		1		:	;	;				:	;	:	;				•	:	:				:		} :	;					;		; ;		
		1979	200	:	;	;	:				;	;	;				:	:	:	:				:	:	:					} ;	;	;		
		1978	žě		:	:				A. A.	, ; ; ;	3.5	5.5	200	25.5	0.0	:	;	;	;				;	:	:	:				:	;	;		
		Pane		. :	145	146	147	2			149	150	151	152			14.3	164	155	156				157	158	159	160				191	162	163	101	l

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Appendix B

Dune July Du 1.1-1.6 Du 1.1-1.6 Du 1.1-1.6 Du 1.1-1.6 Du 1.2 Du 1.2 Du 1.2 Du 1.2 Du 1.3 O.74 O.74 O.79 O.84 O.84 O.84 O.79 O.7
2.3 2.3 3.14 3.14 3.14 3.14 7.7 7.7 7.7 7.7 7.7 7.7 7.7 7.7 7.7 7.
1980 Feb Dr. 1.07 0.81 0.78 0.78 0.79 0.79 0.82 0.79 0.83 0.83 0.83
E ::::
Rov Kov
Aug Aug
July Aug
Ag ::::
March : : : : : : : : : : : : : : : : : : :
1979 Jan 1970 1970 1970 1970 1970 1970 1970 1970
88.28
Panel 165 165 167 167 173 173 174 175 175

